a decisive role in limiting the spread of *A. douglasii,* are questions which remain to be clarified.

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# NUCLEAR CYTOLOGY OF THE CALIFORNIA MOUSE-TAILS (MYOSURUS)[1]

Donald E. Stone

## Introduction

Published accounts of the chromosome numbers in the genus *Myosurus* are limited to three brief reports concerned exclusively with European representatives. In the 1945 edition of the "Chromosome Atlas," a single citation (Gregory, 1941) noted the chromosome number of *M. minimus* as n=8. A check of Gregory's paper, however, reveals that *Myosurus* was one of the few genera in the family for which he had no first hand information. Instead, his citation is based upon the work of Mann (1892) and Hocquette (1922), who found n=8 and 2n=16 respectively. The haploid number was published by Mann as a footnote to his figure 5: "Monaster stage of archesporium, with 8 chromatin segments." Hocquette's account was likewise lacking in details, as his study was part of a general survey of the Ranunculaceae.

The third reference to original work is in the 1955 edition of the "Chromosome Atlas." It is of interest to note that here the earlier citations of Mann and Hocquette are dropped in favor of a more recent

[1] Part of a dissertation submitted to the University of California at Berkeley as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

count by Ehrenberg (1945). Working on Swedish material, Ehrenberg found about 28 chromosomes in the somatic cells. His counts of nine cells showed variations of from 27 to 30 chromosomes, with the best three slides having 28, 28 and 29. He suggests that the Swedish material is tetraploid, being derived from a diploid race with a base number of 7. Hocquette's report of a haploid number of 8 is considered to offer little difficulty as the

TABLE 1. MYOSURUS SPECIMENS CYTOLOGICALLY EXAMINED AND DOCUMENTED.[1]

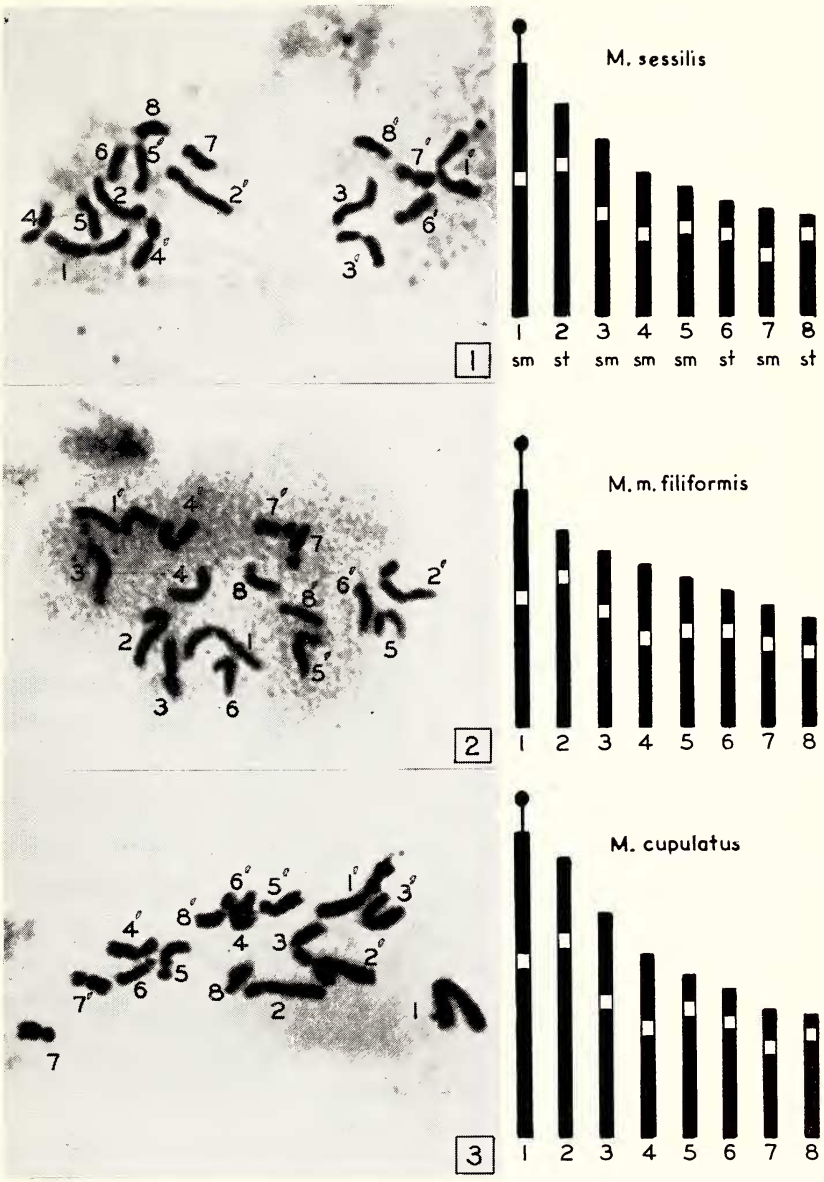
| Collection Data | N | 2N | Fig. |
|---|---|---|---|
| *M. sessilis* Watson | | | |
| *Stone 1(14)*: 5 April 1953, 3 miles east of Maxwell on the Maxwell Road, Colusa County.* | | 16 | 1 |
| *Stone 1(10)*: same data as above. | 8 | | 4 & 5 |
| *M. sessilis* subsp. *alopecuroides* (Greene) Stone | | | |
| *Stone 7(22)*: 10 April 1953, same locality as above. | | 16 | — |
| | 8 | | 6 & 7 |
| *M. minimus* subsp. *apus* (Greene) Campbell | | | |
| *Stone 5(1)*: 10 April 1953, Manning Flat, 5½ miles west of Lower Lake on the road to Kelseyville, Lake County | 8 | | 8 |
| *Stone 5(2)*: same data as above. | 8 | | 9 |
| *H. L. Mason 14275(2)*: 26 April 1952, 5 miles northeast of Crows Landing on the Crows Landing Road, Stanislaus County. | | 16 | — |
| *M. minimus* L. | | | |
| *J. Lid, Stone 73(1)*: 21 June 1955, Hud Island Vestfold County, Norway. | | 16 | 11 |
| *Stone 3(5)*: 4 April 1953, Manning Flat, 5½ miles west of Lower Lake on the road to Kelseyville, Lake County. | 8 | | 10 |
| *H. L. Mason 14501(4)*: 2 April 1953, 3 miles east of Hanford on the road to Visalia, Kings County. | 8 | | — |
| *M. minimus* var. *filiformis* Greene | | | |
| *Stone 7(13)*: 10 April 1953, 3 miles east of Maxwell on the Maxwell Road, Colusa County. | | 16 | 2 |
| *Stone 9(2)*: Spring, 1953, Ajax Field in Willows, Glenn County. | 8 | | 12–14 |
| *M. aristatus* subsp. *montanus* (Campbell) Stone | | | |
| *R. Bacigalupi 4238, Stone 15(5)*: April, 1953, Big Bear Lake, San Bernardino County. | 8 | 16 | — 15 |
| *M. cupulatus* Watson. | | | |
| *T. Robbins 3480, Stone 17 (1)*: 29 April 1952, Providence Mountains, San Bernardino County. | 8 | 16 | 16 3 |

[1] Specimens documenting the chromosome counts have been deposited in the Herbarium of the University of California at Berkeley.

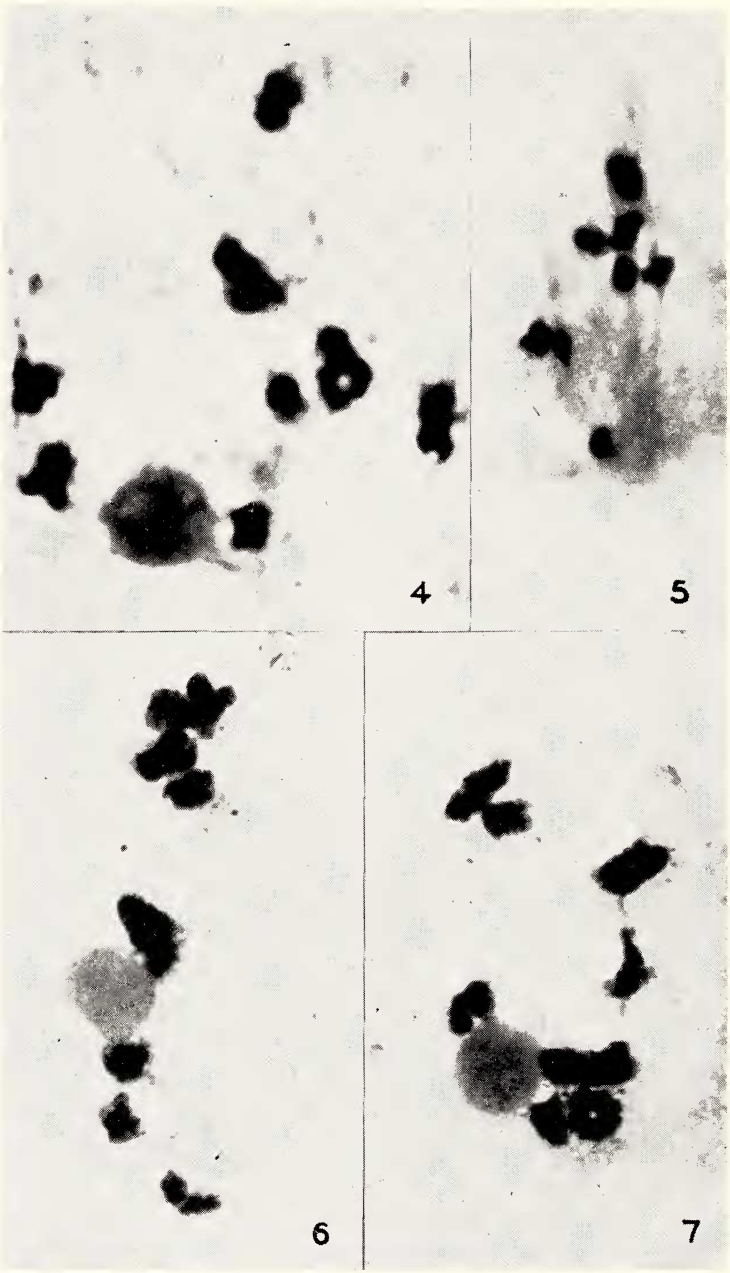
* All localities are in California unless otherwise noted.

closely related genus of *Ranunculus,* which like *Myosurus* has large-Ranunculus-type chromosomes (Langlet, 1932), ranges from n=7, n=8 to n=64 (Darlington and Ammal, 1955).

When the problem of the existence of sympatric biotypes was first suggested (Stone, 1959), it was hoped that cytology might provide some




FIGS. 1–3. Somatic metaphase chromosomes and idiograms of three *Myosurus* species: 1, *M. sessilis,* shoot-apex squash, × 2000; 2, *M. minimus* var. *filiformis,* root-tip squash, × 2200; 3, *M. cupulatus,* root-tip squash, × 1360.




FIGS. 4–7. Meiotic configurations of *M. sessilis* and subspecies: 4, *M. sessilis,* diakinesis, × 1850; 5, *M. sessilis,* metaphase I, × 1850; 6 and 7, *M. sessilis* subsp. alopecuroides, diakinesis, × 1850.

clue to the mechanisms involved in isolation. This hope, unfortunately, was not realized. A survey of chromosome numbers in nine heterogeneous California valley populations (Mason, 1957; Stone, 1957), representatives of two high-mountain species, and a collection from Norway, however, showed only diploid plants with $n = 8$ and $2n = 16$. Although no deviations from the basic number of 8 were found, it is possible that specialized biotypes found in disjunct pools throughout California might prove to be exceptions. Additional information was sought in a karyotype study of three of the most extreme morphological types (figs. 1, 2 and 3). Here again, no differences could be established.

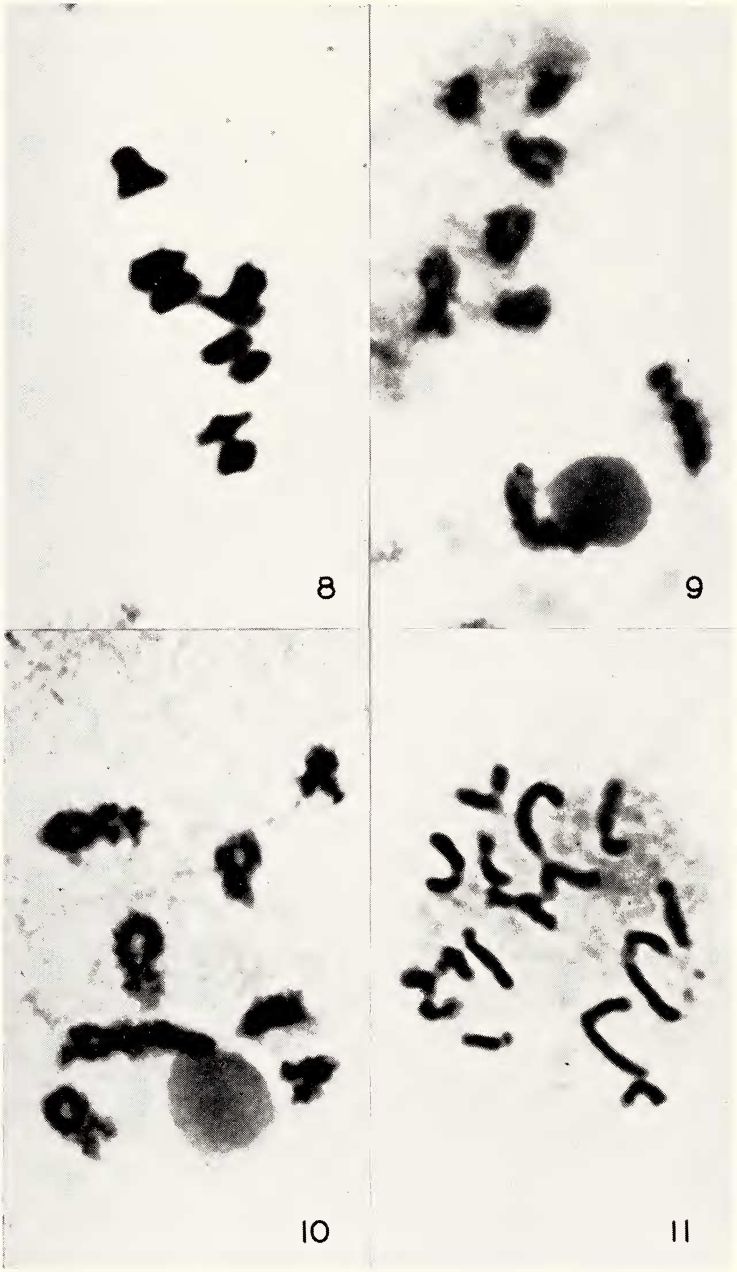
## Material and Methods

All of the material examined cytologically was grown in the Botany Department greenhouse, University of California, Berkeley.

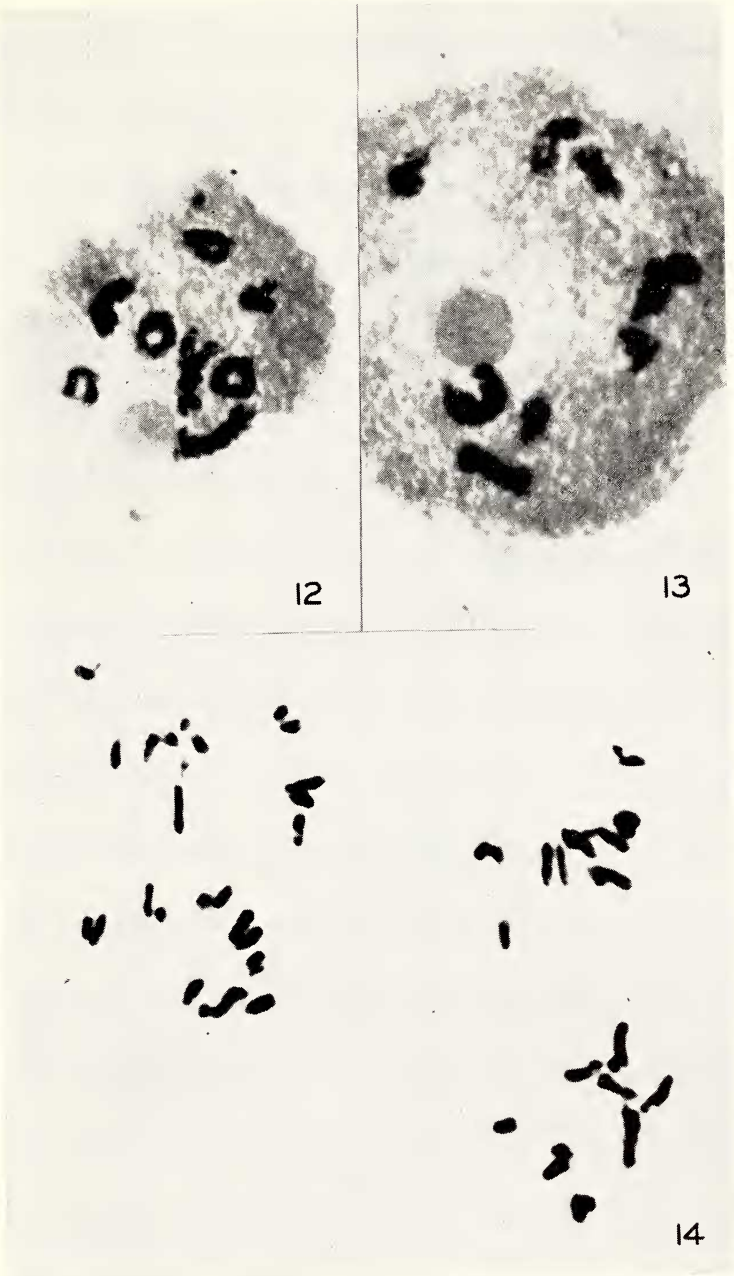
Mitotic stages were most readily obtained from the root tips of young seedlings or from the embryonic tissues of leaf bases and shoot apices. Fixation with acetic-alcohol (1:3) and staining with iron aceto-carmine proved satisfactory in root-tip squashes of young seedlings. Root tips of more mature individuals, however, were extremely difficult to squash, hence special techniques were found necessary. The following four-step process worked well on material examined immediately after squashing: (1) fixation of root tips in acetic-alcohol for 24 hours; (2) pre-staining of the material in aceto-carmine at 60° C. for 2 hours; (3) hydrolyzing in 1N HCl at 60° C. for 1 hour; and (4) washing in distilled water for 15 minutes, after which the material was stored in 70 per cent ethanol. Processed root tips were then squashed using additional iron aceto-carmine stain. Cells hydrolyzed in such a manner have light-stained nucleoli and dark stained chromosomes, and thus are quite favorable for observation of chromosome-nucleolar associations in mitotic prophase. Apparently there is a differential reduction of acidity in the pre-stained nucleus during the hydrolysis (Rattenbury, 1952). Due to the obvious difficulty of chromosome distortion in squashes, paraffin-section methods were tried. However, the minute size of the secondary roots (0.1–0.2 mm. in diameter) and the restricted meristematic region made sectioning efforts fruitless.

Stages of microsporogenesis were used in the study of the meiotic chromosome behavior. When obtained, active pollen mother cells were extremely useful in determining chromosome number, size, and pairing relationships. Three features affecting satisfactory results should be noted: (1) the period of active microsporogenesis; (2) the position of the bud; and (3) the size of the bud and stamens.

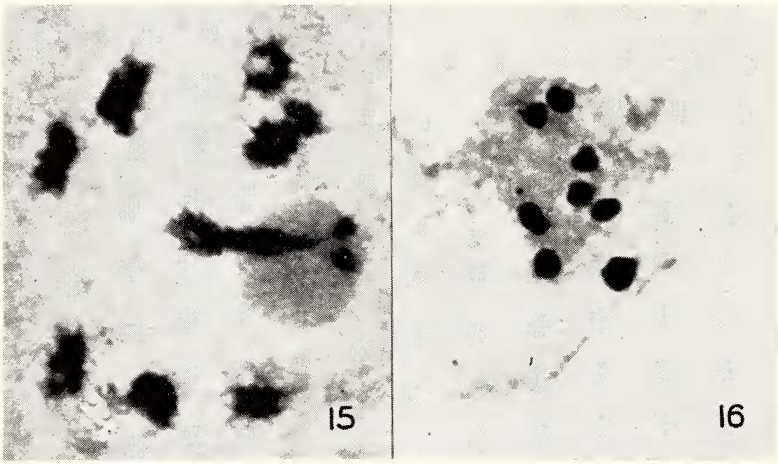
The period of microsporogenesis was found to be of extremely short duration. Out of a total of four or five young buds on a plant it was common to find that most had already matured, while the remainder were premeiotic. Possibly, poor greenhouse conditions were responsible for the shortened meiotic period, but judging from the luxuriant specimens, this




FIGS. 8–11. Meiotic and mitotic configurations of *M. minimus* and subspecies: 8, *M. minimus* subsp. *apus,* metaphase I, × 1850; 9, *M. minimus* subsp. *apus,* diakinesis, × 1850; 10, *M. minimus,* diakinesis, × 1850; 11, *M. minimus,* mitotic metaphase, × 2800.




FIGS. 12–14. Meiosis in *M. minimus* var. *filiformis*: 12–13, diakinesis, × 1850; 14, anaphase II, × 1500.




FIGS. 15–16. Meiosis in two montane species of *Myosurus*: 15, *M. aristatus* subsp. *montanus*, diakinesis showing attachment of chromosome No. 1 (large satellited one) with nucleolus, × 2000; 16, *M. cupulatus*, metaphase I, × 2000.

does not seem likely to be the case. Perhaps a condition such as this, where meiosis occurs at a very early stage and over a short period of time, has a selective advantage in plants that survive in ephemeral environments such as vernal pools.

In all biotypes of *Myosurus* examined, meiosis occurs before peduncle elongation takes place. The meiotic buds are found buried deep in the basal rosette of leaves and peduncles of the more mature flowers. Meiotic buds are usually less than 1.5 mm. in length and hence are extremely difficult to find and remove. Killing and fixing of the entire plant, however, was found practical. Buds were removed under a dissecting microscope at 30×, and flower dissection was completed at 60×, with one stamen (anthers 0.3–0.5 mm. long) at a time being removed for squashing. Plants used for meiotic studies were fixed either in acetic-alcohol or in Linnert's fixative.

The photomicrographs in the accompanying figures were made using a Bausch and Lomb compound microscope having either a 90× (N.A. 1.30) or a 60× (N.A. 1.40) apochromatic objective and a 15× compensating eyepiece. The magnification of each figure is noted in the legend of the figure.

## RESULTS

### Mitotic Chromosome Number and Morphology

Detailed cytological studies were limited to the Manning Flat, Maxwell, and Willows populations (Table 1), but a survey of additional biotypes from the other California Valley populations established a single chromosome number of $2n = 16$. The photographs of figures 1–3, and 11, are representative of the mitotic squashes that were observed in this study. The

generic karyotype consists of 5 submedian and 3 subterminal chromosomes. The largest of the set bears a conspicuous satellite. The idiograms are based on the average of the homologous pairs of chromosomes, as measured in the corresponding photographs. The arbitrary classification of the centromere is based on the relative length of the two arms (Goodspeed, 1945); median (m), arm ratio 1:1; submedian (sm), arm ratio greater than 1:1 but less than 3:1; subterminal (st), arm ratio 3:1 or greater. It is quite apparent that although the satellited chromosome fits in the submedian class, it is very close to the median class, and for all practical purposes it can be considered as such. The conspicuous uniformity in the size gradient from the large satellited chromosome to the smallest subterminal chromosome is common to all three taxa. The most notable difference between the idiograms is the absolute size of the chromosomes. For example, the satellited chromosome is about 6 microns in figure 1, 6 microns in figure 2, and 7 microns in figure 3. As cell size and chromosome size are more or less interdependent and seem to fluctuate considerably in the same plant, no significance was attached to the slight differences in length.

Examination of Norwegian material (fig. 11) has proven the chromosome number to be identical to that of California specimens. Ehrenberg's polyploid counts still remain to be verified.

**Meiotic Chromosome Number and Pairing Relationships**

Diakinesis (figs. 4, 6, 7, 9, 10, 12, 13, and 15) was by far the most common meiotic stage encountered. In part, this occurrence might be attributed to the selected time of fixation: it was found that best results were obtained if fixation was limited to the time between 12 noon and 2 p.m. Infrequently, metaphase I stages (figs. 5, 8, and 16) were found. Later stages in the meiotic sequence were so rare that only two pollen mother cells were observed in the anaphase II stage (fig. 14). The second meiotic anaphase is frequently useful in denoting karyotype differences (Chambers, 1955) and in the case of figure 14 it is possible to verify centromere positions established in mitotic preparations. All of the figures show 8 pairs of chromosomes with no indication of pairing difficulties. It is of interest to note the association of the large satellited chromosome (No. 1) with the nucleolus in the diakinesis figures.

## Summary

Mitotic and meiotic chromosome counts have been made for each of seven taxa of *Myosurus,* on six of which no counts have previously been reported. All examined specimens of the genus *Myosurus* displayed a diploid number of 16, and a haploid number of 8 chromosomes, with no meiotic irregularities.

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## VARIATION IN SECTION TRIGONOPHYLLAE OF NICOTIANA

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Section Trigonophyllae of the genus *Nicotiana* is peculiar to the warm deserts of southwestern North America, and ranges from California to Texas and southward locally as far as Oaxaca. The section, as defined by Goodspeed (1954), includes two species: *N. trigonophylla* Dunal, the range of which coincides with that of the section, and *N. Palmeri* Gray, which is apparently found only in southwestern Utah and western Arizona.

During the course of an ecological investigation of *N. trigonophylla* throughout its range in the United States, the writer encountered facts which cast doubt on the validity of the specific rank of the taxon *N. Palmeri*.

The two members of the section Trigonophyllae are segregated as follows by Goodspeed (1954) in his key and text:

Calyx 8–11 mm. long; corolla 12–23 mm. long, limb 3–4 mm. wide, erect in bud; seed ca. 0.5 mm. long; cauline leaves obtuse to acuminate....*N. trigonophylla*
Calyx 15–17 mm. long; corolla 23–32 mm. long, limb 5–6 mm. wide, oblique in bud; seed ca. 0.7 mm. long; cauline leaves acute to acuminate.....*N. Palmeri*

Both taxa have the same chromosome number (12 pairs) and Kostoff (1943) reported that $F_1$ hybrids between the two show twelve homologous pairs of chromosomes at meiosis.

The writer visited several of the major herbaria[1] of the United States and examined the collections of *Nicotiana,* section Trigonophyllae. Only